Office Action Summary	Application No.	Applicant(s)	
	09/872,493	KENNY ET AL.	
	Examiner	Art Unit	
	Jeanine A Goldberg	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *2/8/02; 6/1/01* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-35* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-35* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *202* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☒ Other: *IDS: 1001* .

DETAILED ACTION

Priority

1. This application claims priority to provisional application 60/209,139, filed June 2, 2000.

Drawings

2. The drawings are acceptable.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

3. Claims 1, 4-5, 11, 13, 20-21, 24, 26 are rejected under 35 U.S.C. 102(e) as being anticipated by Sarto et al (US Pat. 6,022,689, February 2000).

As written, it is unclear what "based on bDNA hybridization" in Claim 1 is intended to mean and has been broadly interpreted to encompass any hybridization assay, since bDNA is based on hybridization.

Sarto et al. (herein referred to as Sarto) teaches a method of in situ hybridization slide processes. As seen in Figure 2a, a slide is used, cells are placed on the top of the slide, labeled DNA probe is added to the slide, a coverslip is placed on the slide, and the slide is simultaneously denatured, hybridized followed by a wash step. Specifically, glass slides are used to immobilize cell samples by allowing the cell solution to dry (limitation of Claim 4)(col.6, lines 55-60). Once the cells were dried, proteinase K (50 ug/ml) was applied to each cell smear and incubated for 10 minutes at 38 degrees C (col. 6, lines 60-68)(limitation of Claim 1a, 20). In the paraformaldehyde fixation method, the cells were treated with proteinase K at 2.5 ug/ml to 10 ug/ml (col. 8, lines 45-50)(limitation of Claim 5). Sarto teaches using DNA FISH in peripheral blood mononuclear cells (limitations of Claim 21). Alternatively fixed or paraffin-embedded tissue sections were deparaffinized and treated with proteinase K and dried on slides (col. 7, lines 1-4)(limitations of Claim 24, 26). Labeled probes in probe solution was added to the slide and allowed to dry (limitations of Claim 1b). The slides were placed in a thermal cycler where the DNA is denatured and hybridize. After hybridization, the coverslips were removed from the glass slides and immersed in NP-40 and NaCl and a detergent(limitations of Claim 1c, 13)(col. 11, lines 15-16). The post hybridization wash was conducted between about 55 and 65 degrees (col. 1,, lines 5-7). There were two washings (col. 7, lines 45-50)(limitations of Claim 11). The hybridization signals in the nuclei were viewed using an inverted fluorescence microscope (limitations of Claim 1d)(col. 7, lines 50-57).

4. Claims 1-4, 6, 9-23, 27-33 are rejected under 35 U.S.C. 102(a) as being anticipated by Antao et al. (Techniques in Quantification and localization of gene expression, page 81-93, June 1999).

Antao et al. (herein referred to as Antao) teaches a method for in situ detection of a nucleic acid analyte within a sample based on bDNA hybridization by a) preparing the sample by immobilizing, permeabilizing using proteinase K b) contacting with a probe wherein at least a portion of the target probe is complementary to at least a portion of the nucleic acid analyte c) washing with a detergent at a temperature of approximately 21 to 60 C and d) detecting the analyte-target probe complex on the substrate (Figure 6.2, page 84)(limitations of Claim 1). Specifically, Antao teaches fixing cells to a slide (page 84-95)(limitations of Claim 20). Antao teaches using a number of cell lines and cell strains including lymphoid cell line, blood cells (limitations of Claims 21-22, 32-33). The microscope slides are incubated in proteinase K (0.5 ug/ml in PBS)(page 85)(limitations of Claim 4). The slides are then contacted with target probes (page 86). The slides are rinsed in wash buffer at room temperature, i.e. between 21 and 60 degrees Celsius (page 86). The hybridized probe is then detected using label probe with a fluorophore directly attached to it or is conjugated to an AP or horseradish peroxidase molecule (page 86)(limitations of Claims 16-17). Antao further teaches incubating the slides with amplifier (limitations of Claim 2). As seen in Figure 6.2, the preamplifier is complementary to a portion of the target probe and forms an analyte-target probe-preamplifier probe complex. Also, an amplifier is hybridized which is complementary to the preamplifier. Moreover, a Ap, fluorescent or chromagenic label is

added to the amplifier probe and the presence of the label is detected. Antao teaches detecting two targets, HIV-1 viral RNA and hn RNP A2/B1 mRNA (limitation of Claim 3). The method of bDNA technology for *in situ* detection which allows for quantification and is specific, reproducible, easy to use and yields results within one day (page 83). Antao teaches using 50ul of preamplifier and amplifier at 0.4 fmol/ul, namely using 20 fmol of preamplifier and amplifier (limitations of Claims 14-15). Antao teaches using 50ul of 2-6 fmol/ul of target probe, namely 0.1-0.3 pmol of target (limitations of Claim 6). The washing solution is comprised of Tris, MgCl2, detergent and ZnCl2 (limitations of Claims 9-10). Antao teaches washing the slide at least twice (page 86)(limitations of Claims 11-12). Each of the wash steps are carried out a room temperature (limitations of Claim 13). Antao teaches centrifuging at 1,500 rpm for 6 minutes (page 85)(limitations of Claim 23). With respect to Claim 18-19, 28, the method of the instant claims and the method steps of Antao are identical. Thus, an inherent property of the method of Antao would be a sensitivity sufficient to detect from 1-2 copies of the nucleic acid analyte.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

5. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of

the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

6. Claims 1-4, 11-13, 16-17, 20-27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Schaeren-Wiemers et al (Histochemistry, Vol. 100, pages 431-440, 1993) in view of Nolte (Advances in Clinical Chemistry, Vol. 33, pages 201-235, 1998) and Decimo (Gene Probes 2: A practical Approach, 1996, pages 183-198) and Xu (In Situ Hybridization, Chapter 4, pages 87-106, 1999).

In the event that the claim is read to require using bDNA hybridization, the following rejection would be applicable to Claim 1. However, as written, it is unclear what "based on bDNA hybridization" is intended to mean and has been broadly interpreted and rejected above.

Schaeren-Wiemers et al. (herein referred to as Schaeren) teaches *in situ* hybridization using digoxigenin-labelled cRNA probes. Schaeren teaches *in situ* hybridization methods for tissue sections and cultured cells using digoxigenin-labelled cRNA probes (abstract). Schaeren teaches mounting on a polylysine coated slide (page 433)(limitations of Claim 4, 20). Schaeren teaches washing in 5xSSC and 0.2 X SSC (page 433, col. 2)(limitations of Claim 11). Schaeren teaches using tissue sections, namely brains and cell cultures, namely optic nerves (page 433, col.

2)(limitations of Claim 21-22, 24-26). Schaeren teaches culturing cells, aspirating off the medium, thereby having used a centrifuge (limitations of Claim 23).

Schaeren does not teach using bDNA hybridization detection means for an in situ hybridization assay. Moreover, Schaeren does not specifically teach using specific concentrations of proteinase K for permeabilizing or washing at temperatures approximately 21-60 degrees Celcius.

However, Nolte teaches branched DNA signal amplification for direct quantitation of nucleic acid sequences in clinical specimens. Nolte emphasizes that the number of target molecules is not altered in the method of bDNA and the resulting signal is directly proportional to the concentration of the target nucleic acid (page 202). The signal of direct hybridization rather than the nucleic acid itself is amplified and thus is directly proportional to the amount of target sequence present in the clinical sample (page 214). BDNA is a nonenzymatic process and is less prone to sample-to-sample variation than with enzymatically mediated target amplification systems. Figure 1 illustrates the bDNA signal amplification assay (limitations of Claims 2). Nolte describes comparisons which were performed between various assays. Nolte teaches that as study by Butterworth teaches the bDNA assay was linear over three orders of magnitude and was the most sensitive assay being approximately 10 times more sensitive than the other assays. Butterworth teaches that the amplifier oligonucleotides enhance the chemiluminescent signal by having multiple branches of the same sequence which bind to the alkaline phosphatase labeled probes which reacts with Dioxetane to produce a chemiluminescent signal which is detected by a luminometer and the concentration of

HBV-DNA is determined (limitations of Claim 16, 17). Nolte teaches using bDNA to detect HCV, for example (page 206)(limitations of Claim 3). Nolte suggests the application for bDNA in *in situ* hybridization assays (page 231).

Moreover, Decimo et al. provides basic conditions for conducting *in situ* hybridization assays. Decimo teaches pre-hybridization treatments of slides with proteinase K, 1 ug/ml (page 192). The hybridization is performed followed by washing steps. The post-hybridization washes were performed at 55 C for about 45 minutes (limitations of Claim 1cm 13). Decimo teaches that approximately 5-10 ul of hybridization mixture is required per cm2 of tissue section. Decimo teaches several wash steps following hybridization (page 195)(limitations of Claim 11-12).

Claim 27 requires that the position of a nucleic acid analyte within a sample is determined as indicative of the position of the nucleic acid analyte in the cell. Thus, the mere detection of the nucleic acid within the cell would be indicative of the position of the nucleic acid within the cell. Thus, based upon the language of the claim, the claim only requires identifying the complex within a cell.

Therefore, it would have been prima facie obvious to one of ordinary skill at the time the invention was made to have modified the *in situ* hybridization assay using digozxigenin-labelled cRNA probes with the improved bDNA hybridization method. The skilled artisan would have recognized that a comparison of the ELISA-based cheiluminescent method of Schaeren could be modified and improved by substituting the ELISA-based chemiluminescent method with bDNA methods, as taught by Nolte to be more sensitive, precise and linear. Given the comparison of solution hybridization

methods taught by Nolte, there would have been a reasonable expectation that the bDNA method would have been more sensitive, precise and linear *in situ*. Hybridization mechanisms *in situ* and in solution are analogous, thus, the ordinary artisan would have expected bDNA to act superiorly to ELISA-based chemiluminescent methods *in situ*, as exemplified by the in solution comparison. *In situ* hybridization detection of probes would be enhanced by signal amplification such as bDNA. The signal amplification would allow for greater luminescence and greater detection signals.

With respect to the specific conditions for performing *in situ* hybridization, the skilled artisan would have been motivated to have optimized the conditions. As noted in *In re Aller*, 105 USPQ 233 at 235, "More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." Routine optimization is not considered inventive and no evidence has been presented that the probe selection performed was other than routine, that the products resulting from the optimization have any unexpected properties, or that the results should be considered unexpected in any way as compared to the closest prior art. Thus, performing the *in situ* hybridization with conditions provided by Decimo as optimal for ISH, would have been within the optimization of the ordinary artisan. Therefore, using particular concentrations and temperatures would have constituted optimization on the part of the ordinary artisan.

7. Claims 6-10 are rejected under 35 U.S.C. 103(a) as being unpatentable over Schaeren-Wiemers et al (Histochemistry, Vol. 100, pages 431-440, 1993) in view of

Nolte (Advances in Clinical Chemistry, Vol. 33, pages 201-235, 1998) and Decimo (Gene Probes 2: A practical Approach, 1996, pages 183-198) as applied to Claims 1-4, 11-13, 16-17, 20-27 above, and in further view of Xu (In Situ Hybridization, Chapter 4, pages 87-106, 1999).

Neither Schaeren, Nolte nor Decimo specifically teach particular concentrations of target probe or particular washing conditions.

However, Similarly, Xu teaches *in situ* hybridization of mRNA with hapten labeled probes (page 87). Haptenized nucleotides include biotin, digoxigenin, biotin. Xu also provides conditions for hybridization, washing, and detection of probe (page 95). The washing steps are performed at 55-65 degrees Celcius. Xu teaches that "for many probes, 55 degrees Celsius is best since stronger signals are obtained (page 95). Xu teaches that 0.1-0.5 ug of probe is used for hybridization. Xu teaches several washing solutions which use Triton, NaCl and KCl (limitations of Claim 7-10).

With respect to Claim 6, Schaeren does not specifically teach using approximately 0.1 pmoles to 10 pmoles of the target probe. Schaeren teaches preparing hybridization mixture by adding 200 ng cRNA per ml hybridization buffer. Moreover, 200 ul hybridization buffer was used. However, Xu teaches average *in situ* probes are between 32-36 nucleotides in length. Thus, using a 34 nucleotide probe as an average length, 130 as the average molecular weight of each base, the probe is approximately 4,420 g/mol. Thus, there is approximately .91 pmoles of target probe used. Thus, performing the *in situ* hybridization with various length probes, probes of various sequences, would have been within the optimization of the ordinary artisan.

Therefore, using particular concentrations and temperatures would have constituted optimization on the part of the ordinary artisan.

With respect to the specific conditions for performing *in situ* hybridization, the skilled artisan would have been motivated to have optimized the conditions. As noted in *In re Aller*, 105 USPQ 233 at 235, "More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." Routine optimization is not considered inventive and no evidence has been presented that the probe selection performed was other than routine, that the products resulting from the optimization have any unexpected properties, or that the results should be considered unexpected in any way as compared to the closest prior art. Thus, performing optimizing the *in situ* hybridization with conditions provided by Xu as optimal for ISH, would have been within the optimization of the ordinary artisan. Therefore, using particular concentrations and temperatures would have constituted optimization on the part of the ordinary artisan.

8. Claim 5 is rejected under 35 U.S.C. 103(a) as being unpatentable over Schaeren-Wiemers et al (Histochemistry, Vol. 100, pages 431-440, 1993) in view of Nolte (Advances in Clinical Chemistry, Vol. 33, pages 201-235, 1998) and Decimo (Gene Probes 2: A practical Approach, 1996, pages 183-198) and Xu (In Situ Hybridization, Chapter 4, pages 87-106, 1999) and in further view of Sarto et al (US Pat. 6,022,689, February 2000).

Neither Schaeren, Nolte nor Decimo specifically teach permeabilizing with about 5-20 ug/ml of Proteinase K.

However, Sarto et al. (herein referred to as Sarto) teaches a method of in situ hybridization slide processes. As seen in Figure 2a, a slide is used, cells are placed on the top of the slide, labeled DNA probe is added to the slide, a coverslip is placed on the slide, and the slide is simultaneously denatured, hybridized followed by a wash step. Specifically, glass slides are used to immobilize cell samples by allowing the cell solution to dry (limitation of Claim 4)(col.6, lines 55-60). In the paraformaldehyde fixation method, the cells were treated with proteinase K at 2.5 ug/ml to 10 ug/ml (col. 8, lines 45-50)(limitation of Claim 5).

Therefore, it would have been prima facie obvious to one of ordinary skill at the time the invention was made to have used about 5-20 ug/ml of Proteinase K for permeabilizing cells prior to *in situ* hybridization. With respect to the specific conditions for performing *in situ* hybridization, the skilled artisan would have been motivated to have optimized the conditions. As noted in *In re Aller*, 105 USPQ 233 at 235, "More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." Routine optimization is not considered inventive and no evidence has been presented that the probe selection performed was other than routine, that the products resulting from the optimization have any unexpected properties, or that the results should be considered unexpected in any way as compared to the closest prior art. Thus, performing the *in situ* hybridization with conditions provided by Sarto as optimal for ISH,

would have been within the optimization of the ordinary artisan. Therefore, using particular concentrations and temperatures would have constituted optimization on the part of the ordinary artisan.

9. Claims 14-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Schaeren-Wiemers et al (Histochemistry, Vol. 100, pages 431-440, 1993) in view of Nolte (Advances in Clinical Chemistry, Vol. 33, pages 201-235, 1998) and Decimo (Gene Probes 2: A practical Approach, 1996, pages 183-198) and in further view of Kern et al. (J. Clin. Microbiol. Vol. 34, No. 12, pages 3196-3202, 1996).

Neither Schaeren, Nolte, Decimo, nor Xu specifically teaches using between 1 fmol and 10pmoles of preamplifier or amplifier.

Kern teaches a method of bDNA quantification of HIV. The method of kern teaches using .70 fmol of preamplifier per ul and 1.0 fmol of bDNA amplifier per ul. The 50ul of solution was used.

Therefore, it would have been prima facie obvious to one of ordinary skill at the time the invention was made to have used the specified conditions provided by Kern in the absence of specific conditions provided in Nolte for the amount of preamplifier and amplifier used in a bDNA assay. The ordinary artisan would have looked to the art to the ranges and units of preamplifier and amplifier used in bDNA assays to optimize the method of using bDNA and in situ hybridization. Thus, using approximately 1 fmol to about 10pmoles of amplifier and of preamplifier, would have been obvious to the ordinary artisan at the time the invention was made.

10. Claims 18-19 are rejected under 35 U.S.C. 103(a) as being unpatentable over Schaeren-Wiemers et al (Histochemistry, Vol. 100, pages 431-440, 1993) in view of Nolte (Advances in Clinical Chemistry, Vol. 33, pages 201-235, 1998) and Decimo (Gene Probes 2: A practical Approach, 1996, pages 183-198) or Xu (In Situ Hybridization, Chapter 4, pages 87-106, 1999) as applied to Claims 1-4, 6-13, 16-17, 20-27 above, and further in view of Siadat-Pajouh (J. of Hist. And Cytochemistry. Vol. 42, No. 11, pages 1503-1512, 1994).

Neither Schaeren, Nolte, Decimo, nor Xu specifically teach the method has a sensitivity to detect from 1-2 copies of the nucleic acid.

However, Siadat-Pajouh et al. (herein referred to as Siadat) teaches a method of detecting single-copy HPV genomic *in situ* (limitations of Claim 29). The method of Siadat is a highly sensitive and quantitative fluorescence-based *in situ* hybridization technique that can detect as few as one to five copies in the HPB cells using digoxygenin tail-labeled oligonucleotides (limitations of Claims 28, 30). The method is quick and may be carried out in four and half hours (abstract). Siadat uses human cervical carcinoma cell lines (page 1504, col. 1)(limitations of claims 31-33) Siadat teaches that the commonly used non-isotopic ISH methods are based on labeling probes with biotin or digoxigenin. The signal can be amplified in various ways and detected either via an enzyme substrate or binding to a fluorochrome. Siadat teaches that the advantage of the enzyme substrate detection system is that the intensity of the signal increases as function of time, making it more sensitive compared with other

systems (page 1503, col. 2). Siadat teaches that much of the optimization of *in situ* hybridization conditions is necessarily empirical in nature.

Therefore, it would have been prima facie obvious to one of ordinary skill at the time the invention was made to have performed the *in situ* hybridization method of Schaeren in view of Nolte with the optimized conditions of Decimo or Xu and further have optimized the *in situ* hybridization assay to detect a single copy of the nucleic acid analyte in a cell, as taught by Siadat. Siadat teaches the importance of detecting single copy nucleic acids and provides means by which the *in situ* hybridization may be performed. The ordinary artisan would have been motivated to have optimized the in situ hybridization assay to include detection of single copy targets.

11. Claims 28-35 are rejected under 35 U.S.C. 103(a) as being unpatentable over Siadat-Pajouh (J. of Hist. And Cytochemistry. Vol. 42, No. 11, pages 1503-1512, 1994) in view of Nolte (Advances in Clinical Chemistry, Vol. 33, pages 201-235, 1998).

Siadat-Pajouh et al. (herein referred to as Siadat) teaches a method of detecting single-copy HPV genomic *in situ* (limitations of Claim 29). The method of Siadat is a highly sensitive and quantitative fluorescence-based *in situ* hybridization technique that can detect as few as one to five copies in the HPB cells using digoxygenin tail-labeled oligonucleotides (limitations of Claims 28, 30). The method is quick and may be carried out in four and half hours (abstract). Siadat uses human cervical carcinoma cell lines (page 1504, col. 1)(limitations of claims 31-33). Siadat teaches that the method of FISH would be useful in detection and quantitation of a variety of viral genomes, oncogenes

and drug resistant genes, in a variety of morphologically intact cells and tissues (limitations of Claims 31, 34). Siadat teaches that the commonly used non-isotopic ISH methods are based on labeling probes with biotin or digoxigenin. The signal can be amplified in various ways and detected either via an enzyme substrate or binding to a fluorochrome. Siadat teaches that the advantage of the enzyme substrate detection system is that the intensity of the signal increases as function of time, making it more sensitive compared with other systems (page 1503, col. 2). Siadat teaches that much of the optimization of *in situ* hybridization conditions is necessarily empirical in nature.

Siadat does not specifically teach using bDNA for signal amplification and detection of nucleic acid analytes *in situ*.

However, Nolte teaches branched DNA signal amplification for direct quantitation of nucleic acid sequences in clinical specimens. Nolte emphasizes that the number of target molecules is not altered in the method of bDNA and the resulting signal is directly proportional to the concentration of the target nucleic acid (page 202). The signal of direct hybridization rather than the nucleic acid itself is amplified and thus is directly proportional to the amount of target sequence present in the clinical sample (page 214). BDNA is a nonenzymatic process and is less prone to sample-to-sample variation than with enzymatically mediated target amplification systems. Figure 1 illustrates the bDNA signal amplification assay (limitations of Claims 2). Nolte describes comparisons which were performed between various assays. Nolte teaches that as study by Butterworth teaches the bDNA assay was linear over three orders of magnitude and was the most sensitive assay being approximately 10 times more sensitive than the other assays.

Butterworth teaches that the amplifier oligonucleotides enhance the chemiluminescent signal by having multiple branches of the same sequence which bind to the alkaline phosphatase labeled probes which reacts with Dioxetane to produce a chemiluminescent signal which is detected by a luminometer and the concentration of HBV-DNA is determined (limitations of Claim 16, 17). Nolte teaches using bDNA to detect HCV, for example (page 206)(limitations of Claim 3). Nolte suggests the application for bDNA in *in situ* hybridization assays (page 231).

Therefore, it would have been prima facie obvious to one of ordinary skill at the time the invention was made to have modified the *in situ* hybridization assay using digozxigenin-labelled cRNA probes and signal amplification with the improved bDNA signal amplification hybridization method. The skilled artisan would have recognized that a comparison of the ELISA-based cheiluminescent method of Siadat could be modified and improved by substituting the ELISA-based chemiluminescent method with bDNA methods, as taught by Nolte to be more sensitive, precise and linear. Given the teachings of Siadat that signal amplifications may be performed in various ways, and comparison of several hybridization methods, there would have been a reasonable expectation that the bDNA method would have been more sensitive, precise and linear in situ. *In situ* hybridization detection of probes would be enhanced by signal amplification such as bDNA. The signal amplification would allow for greater luminescence and greater detection signals. Thus, the ordinary artisan would have been motivated to have used the signal amplification method of bDNA in the signal amplification method taught by Siadat because the skilled artisan would have

recognized that the bDNA signal amplification method is a quantitative method which is very sensitive.

With respect to Claim 35, depending upon the sample available the ordinary artisan would have been motivated to have used tissue samples as opposed to cells for analyzing the nucleic acids *in situ*. Therefore, using the methods taught in the art for preparation of tissue samples, would have been obvious to the skilled artisan at the time the invention was made. With specific respect to the tissue types recited in Claim 35, the skilled artisan would have been motivated to have used any of the tissue types provided depending upon the sample available for analyses. In situ hybridization has been used on biopsies from cancer patients, thus, any tissue biopsy which requires analyzation would be appropriate and the ordinary artisan would be motivated to analyze the biopsy tissue.

Conclusion

12. No claims allowable over the art.

13. Any inquiry concerning this communication or earlier communications from the examiner should be directed to examiner Jeanine Goldberg whose telephone number is (703) 306-5817. The examiner can normally be reached Monday-Friday from 8:00 a.m. to 5:30 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones, can be reached on (703) 308-1152. The fax number for this Group is (703) 305- 3014.

Any inquiry of a general nature should be directed to the Group receptionist whose telephone number is (703) 308-0196.

J. Goldberg

Jeanine Goldberg
Patent Examiner
[redacted]
October 1, 2003